THE DEVELOPMENT OF THE SPORES IN PLEURAGE ZYGOSPORA

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(WITH PLATE XIX)

Pleurage zygospora (Speg.) Kuntze, formerly reported from Italy alone, has been shown by the cultural methods employed by THAXTER and later by GRIFFITHS[1] to occur throughout this country. This species was first described by SPEGAZZINI[2] in 1878, and was placed in *Sordaria* as *S. zygospora*. It was later transferred from this genus by SACCARDO[3] and placed in *Philocopra*. This transfer was made on the basis of the apparent number of spores being 16 in place of 8. Later (1898) KUNTZE[4] described it as a *Pleurage*. This classification was retained by GRIFFITHS in his memoir. The species name *zygospora* has been retained throughout.

The position of this species depends upon the interpretation which is placed upon the mature ascospore. It is obvious that such an interpretation should be based upon a knowledge of the development of the spore. A cytological study of the peculiar appendaged condition of the spores in the genus *Pleurage* has not, so far as the writer is aware, been carefully made. A complete understanding of the nature of the primary appendages necessarily involves a study of the behavior of the nucleus of the primary sporogenous cell. This detail was not attempted by GRIFFITHS in his memoir on the Sordariaceae.

The spores of the species of this genus in which the primary appendage occurs are known to pass through a peculiar mode of development. The primary sporogenous cell is cut out of the cytoplasm of the ascus after the method described by HARPER.[5] These

[1] GRIFFITHS, D. A., The North American Sordariaceae. Mem. Torr. Bot. Club 11:1–134. 1901.

[2] SPEGAZZINI, C., Michelia 1:227. 1878.

[3] SACCARDO, P. A., Sylloge Fungorum 1:251. 1882.

[4] KUNTZE, OTTO, Revisio Generum Plantarum 3:505. 1898.

[5] HARPER, R. A., Annals of Botany 13:507. 1899.

cells are quite small, cylindrical, hyaline, straight or curved, and do not differ markedly in structure from the abundant epiplasm of the young ascus. They grow for a time and become transformed into filamentous cells, the length of which varies in different species. When one of these cells approximates its maximum length, the upper end begins to enlarge and forms an ellipsoid portion into which the greater part of the protoplasm migrates. This portion then becomes cut off by a cross-wall and forms the fertile cell of the spore. The remainder constitutes the so-called primary appendage. *Pleurage zygospora* shows upon a superficial examination that the spores are developed after this same general method.

Because of the peculiar interest attaching to this method of spore-formation, and also because of the great variability found to obtain in spores of *Pleurage zygospora* from collections made in the vicinity of Austin, Texas, the writer deemed it desirable to make a detailed cytological study of their complete development. The material for this study was grown in damp chambers on the natural substratum, and was prepared according to the methods employed in modern cytological technique. Flemming's solution, weaker formula, as a fixing agent left nothing to be desired. The best stain was found to be the Flemming triple stain. Sections 10 μ thick gave very good preparations for study.

The immature perithecia contain many different stages in the development of the ascus, so that the stages could be studied in unbroken continuity in any one section. The study of fixed and stained material was supplemented by the use of fresh material crushed in water on the slide, and then treated with dilute aqueous gentian violet or eosin glycerin.

The details of nuclear division in the ascus and the young spore were not studied critically, as it did not appear that the material was favorable for a determination of the phenomena of chromosome reduction. The primary nucleus of the young ascus is quite large, and the chromatin material appears to be divided into four parts, but spindle stages were not observed (figs. 1–3). The cytoplasm is quite vacuolate, and this condition increases with the rapid enlargement of the ascus (fig. 4). After the ascus reaches about one-half its mature size, the division of the primary nucleus

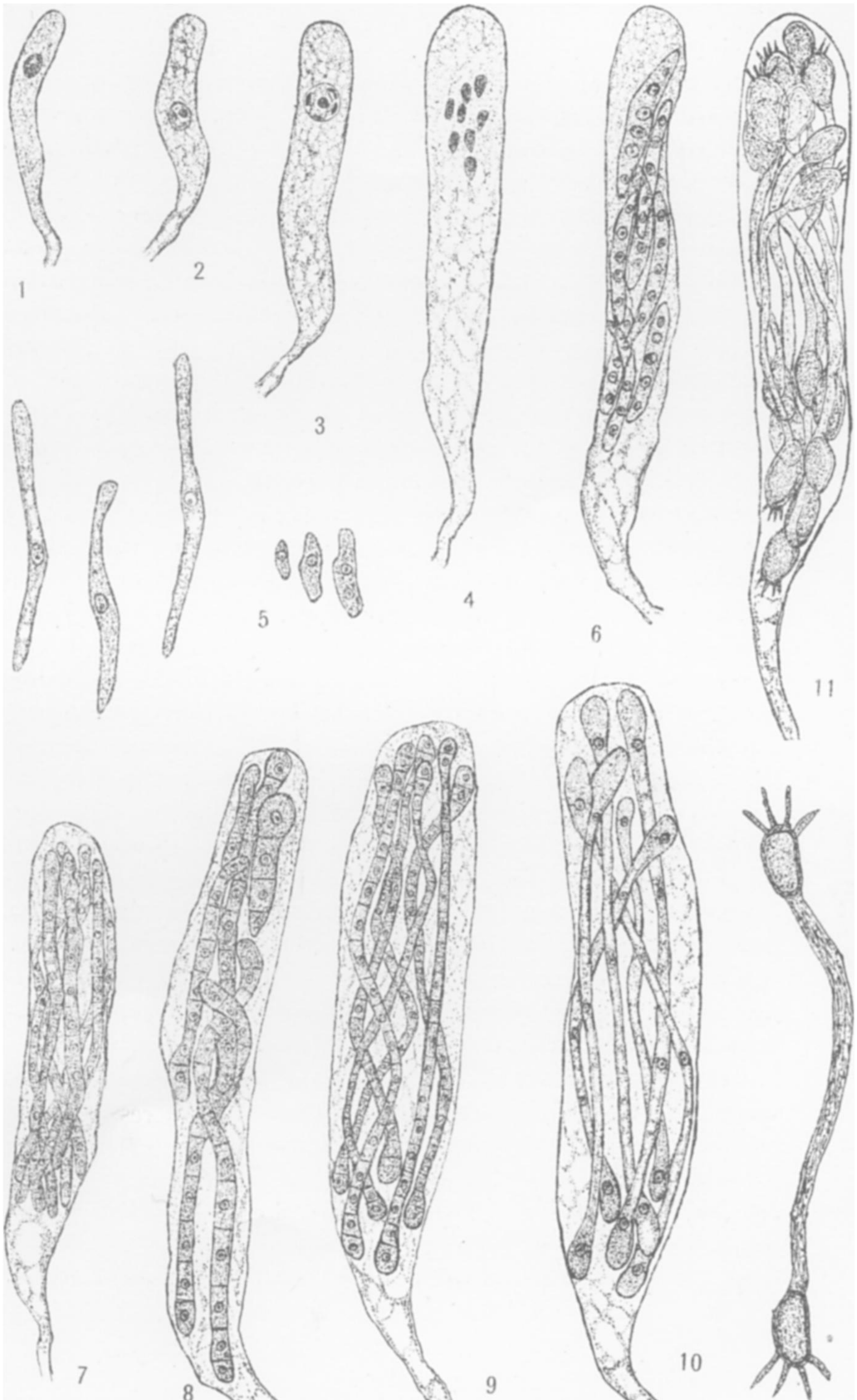
takes place, and this is followed quickly by two succeeding divisions, so that 8 cells are formed in the ascus in the usual way. These cells lie quite close together near the upper end of the ascus, are very small, and there is an abundant epiplasm (fig. 4). These are the primary sporogenous cells which later give rise to the fertile cells of the spores. They are cylindrical, hyaline, and the cytoplasm is more dense than the epiplasm surrounding them. The ascus has not at this time reached its mature size, and the spores appear as very minute cells in its upper end (fig. 4).

Further development from the primary cells was found to be quite variable in any one perithecium. In all cases, however, the sporogenous cell begins to elongate and to enlarge in all directions. It most frequently happens that the growth takes place faster on one side than on the other, so that the cell becomes somewhat crescentic in shape. It is also slightly bulged out near its middle (figs. 5, 6). It often happens that the nucleus of the young spore divides before the cell has grown to more than two or three times its original length (fig. 5), but this is by no means always the case. Frequently the nucleus remains undivided even after the cell has reached the length equal to half that of the ascus. In a few cases it was observed that the nucleus did not divide at all. As the cell elongates, its cytoplasm becomes vacuolate and more resembles the epiplasm from which it is derived. The nucleus usually divides, and after the daughter nuclei have moved apart, succeeding divisions take place until a number of free nuclei are present in the spore (figs. 6–10). The filamentous cell grows in length until in many cases it becomes longer than the ascus, and is consequently compelled to assume a spiral position. It is at this time almost uniform in diameter throughout its length, and the nuclei are distributed along the entire filament. The cytoplasm contains many vacuoles and the epiplasm is greatly reduced. The formation of cross-walls may or may not take place. Both types of asci occur in the same perithecium, but one type usually predominates in any one perithecium. In the cross-walled type the walls are usually formed at about the stage shown in fig. 6. Stages such as shown in figs. 10 and 11 never form cross-walls. In some perithecia there may be only a single ascus of the cross-walled type, while in others

this type predominates. In no instance has an ascus been observed in which both types occur. In all of the multicellular filaments, each cell has a nucleus, and the cytoplasm is apparently more dense than in the other type.

About the time the filament has reached its maximum length or slightly earlier, the two ends begin to enlarge, and the cytoplasm in these ellipsoid portions becomes very dense. Each end portion usually contains a single nucleus (fig. 10). No cases were observed in which two nuclei were present, but occasionally it appears that a nucleus does not migrate to the end of the filament, and in such cases the end portion becomes abortive. In the case of the multicellular type, the end portion which is enlarged may consist of a single cell or of two or more cells (figs. 8, 9). These enlarged end portions become the fertile cells of the spore, and at maturity are about $15-20 \times 25-40\,\mu$ in size. The connecting filament is homologous in origin with the primary appendage of other species of the genus. This primary appendage is seen to connect the two fertile portions of a single spore in this species. Each of these fertile portions functions as a spore. The primary connecting filament persists for some time, but at the maturity of the perithecium and the shedding of the spores, it has almost disappeared, thus separating the two portions, and the ascus produces, therefore, the functional equivalent of 16 spores. The primary appendage in this species is either one multinucleate cell or a filament made up of a number of cells. In some cases the multicellular spores were observed to present a somewhat abortive appearance (fig. 8), in which case it happens that one fertile cell only is produced. Abortion is not uncommon in the other type of spore, so that often only five or six pairs of fertile cells are produced, or sometimes the number of fertile cells is different in the two ends of the ascus.

The question quite naturally arises as to the definition of a spore, and whether this species produces 8 or 16 spores. It was on this basis that SACCARDO transferred the species to *Philocopra*. Functionally 16 spores are produced, but morphologically there are only 8. These 8 spores are either three-celled, that is, two fertile cells connected by a long multinucleate cell (fig. 10), or they may be multicellular, consisting of two fertile parts connected by



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a long multicellular sterile portion which eventually disappears. The basis of the structure and origin of the spores seems to me to be the proper basis for their definition. I regard this species, therefore, as eight-spored, and the classification of KUNTZE as the proper one.

The question concerning the probable significance of the two types of spores produced, while interesting, must remain more or less speculative. It seems probable that this condition must have been derived from ancestors which produced multicellular spores, and that the production of fertile and sterile cells is due to specialization and sterilization. The first step in this process might have consisted in the formation of the enlarged fertile cells, following which the connecting cells ceased to be functional. The loss of cross-walls and the consequent production of the unicellular appendaged type of spore, characteristic of this and other members of the Sordariaceae, could easily follow.

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EXPLANATION OF PLATE XIX

All figures were drawn from sections with the aid of a camera lucida, a Leitz $\frac{1}{12}$ oil immersion lens, and ocular no. 1, and are magnified 880 times.

FIGS. 1–3.—Young asci showing relatively large nuclei and rather dense cytoplasm.

FIG. 4.—Ascus about two-thirds mature size, with vacuolate epiplasm and 8 primary sporogenous cells.

FIG. 5—Various stages in the growth of the spore filament.

FIG. 6.—Young spores showing the crescentic shape and with several nuclei; the ascus has not increased much in size.

FIG. 7.—Spore filaments of the cross-walled type; the spores are almost as long as the ascus, but the end portions have not begun to increase in size; this same appearance prevails in the other type of spores.

FIG. 8.—A later stage than fig. 7; some of the spores are abortive, a condition which frequently occurs.

FIG. 9.—Older stage of the same type of spore shown in fig. 8, with the ends becoming enlarged.

FIG. 10.—The unicellular type, showing the multinucleate condition and enlarged ends, each containing a single nucleus.

FIG. 11.—Mature spores of the type shown in fig. 10; secondary appendages derived from the epiplasm are shown.

FIG. 12.—Mature spore of the three-celled type.